

Laboratory-Based Cryogenic Soft X-ray Tomography and Correlative Microscopy: 3D Visualization Inside the Cell

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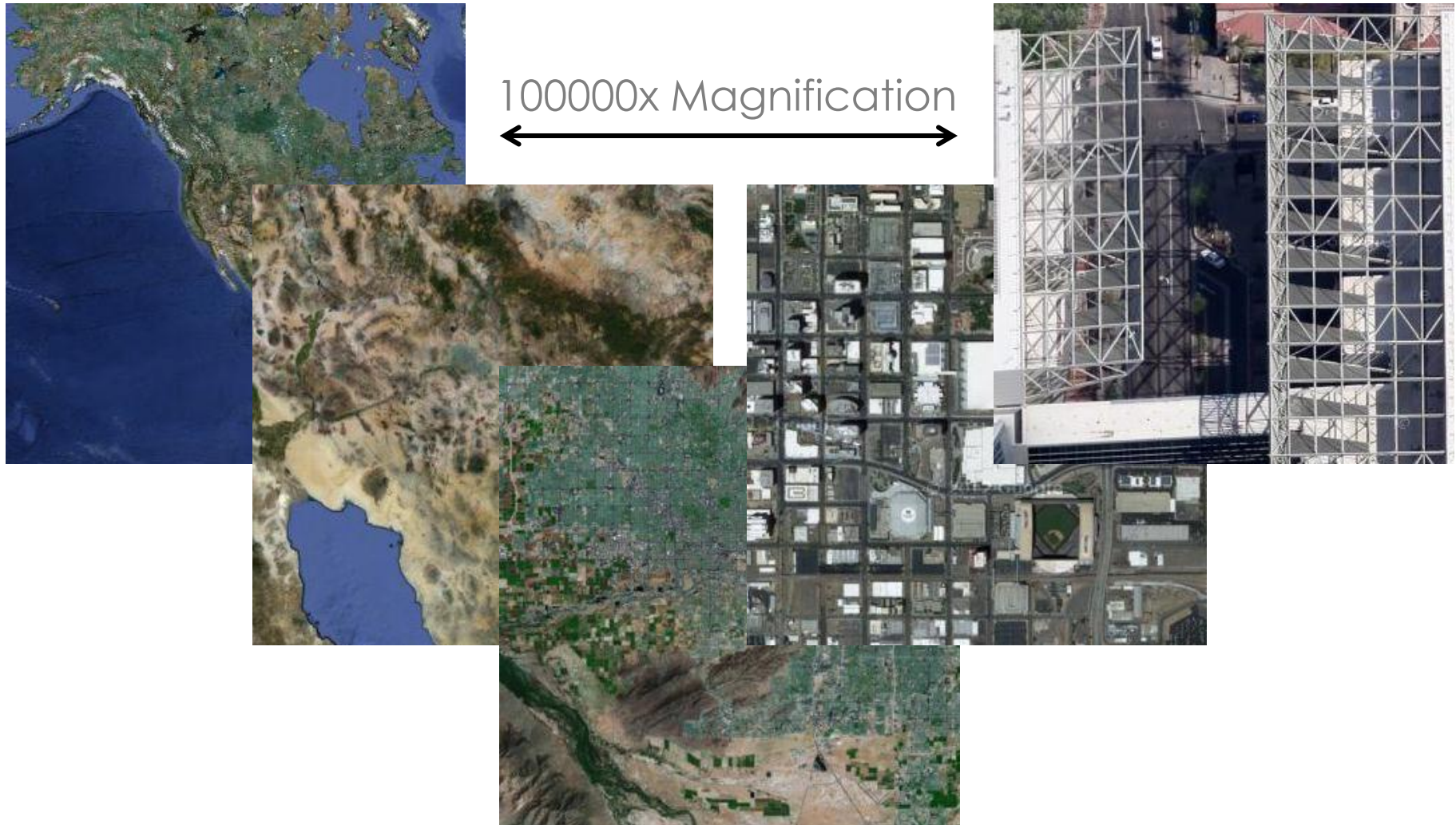
Support from:



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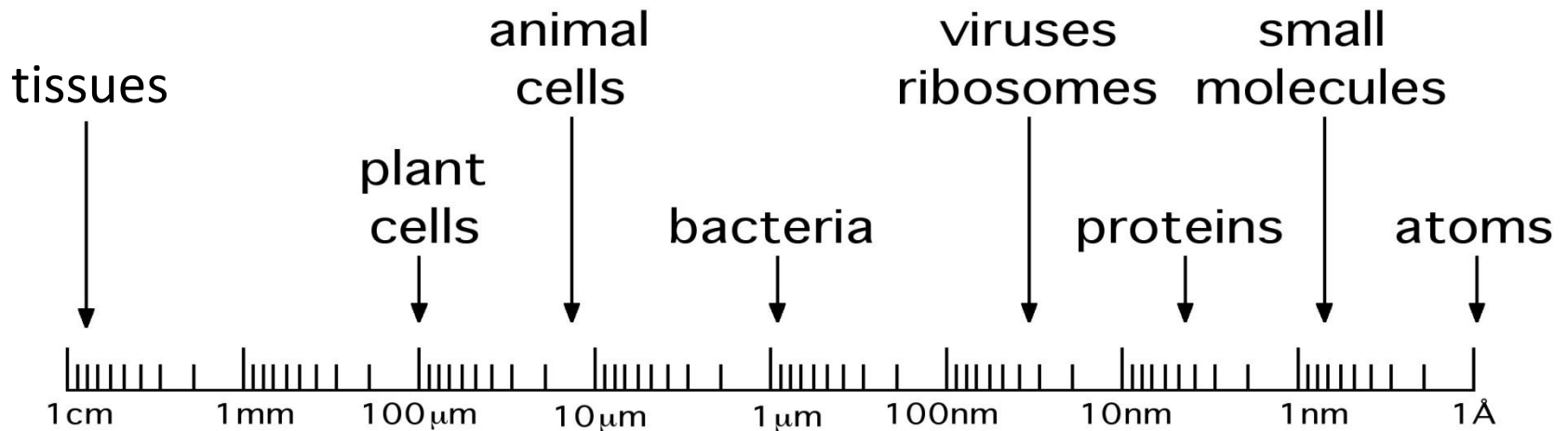
EMSL is located at PNNL

Need For Multiscale Imaging



Biology shares similar range of relevant spatial scales!

Length Scales of Biology and Imaging Capabilities



fluorescent microscopy

Can't image unlabeled structures

electron microscopy

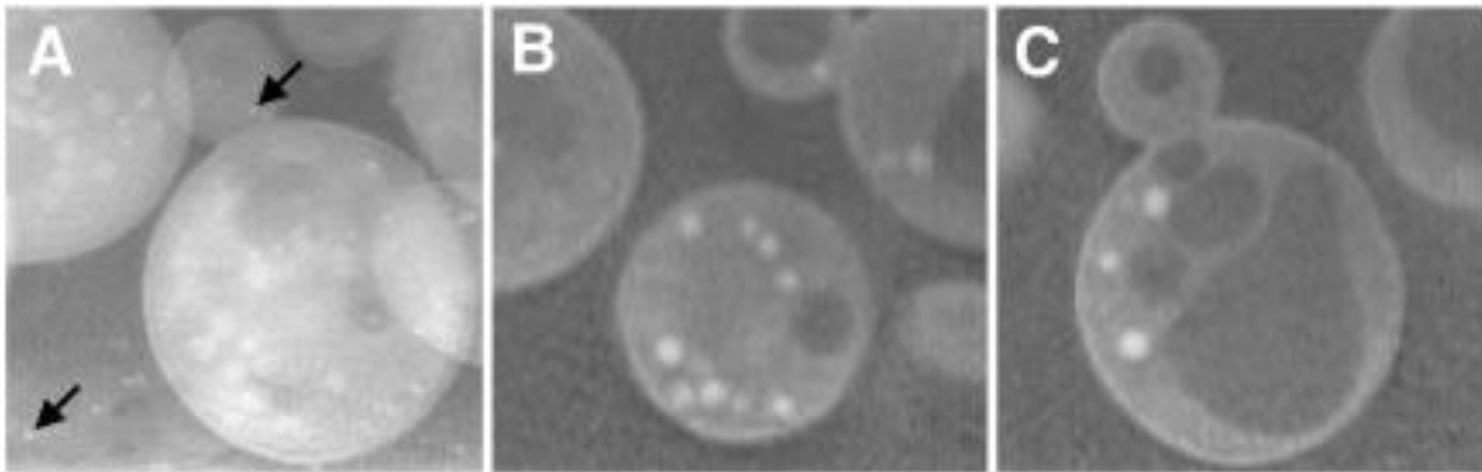
Small field of view but high resolution for thicknesses up to 500 nm.

x-ray tomography

Whole cell context without sectioning but need access to synchrotron source

x-ray diffraction

Synchrotron Generated Tomogram of Frozen-Hydrated Yeast Cells



Larabell, C.A. and Le Gros, M.A. (2004) *Mol. Biol. Cell.* 15:957-962

Imaged in frozen hydrated state within 10 μm glass capillary tube
at a total cumulative imaging dose of 100 – 1,000 MGy and
spatial resolution of 60 nm.

Can we develop a laboratory-based platform
for soft x-ray tomography of whole cells?

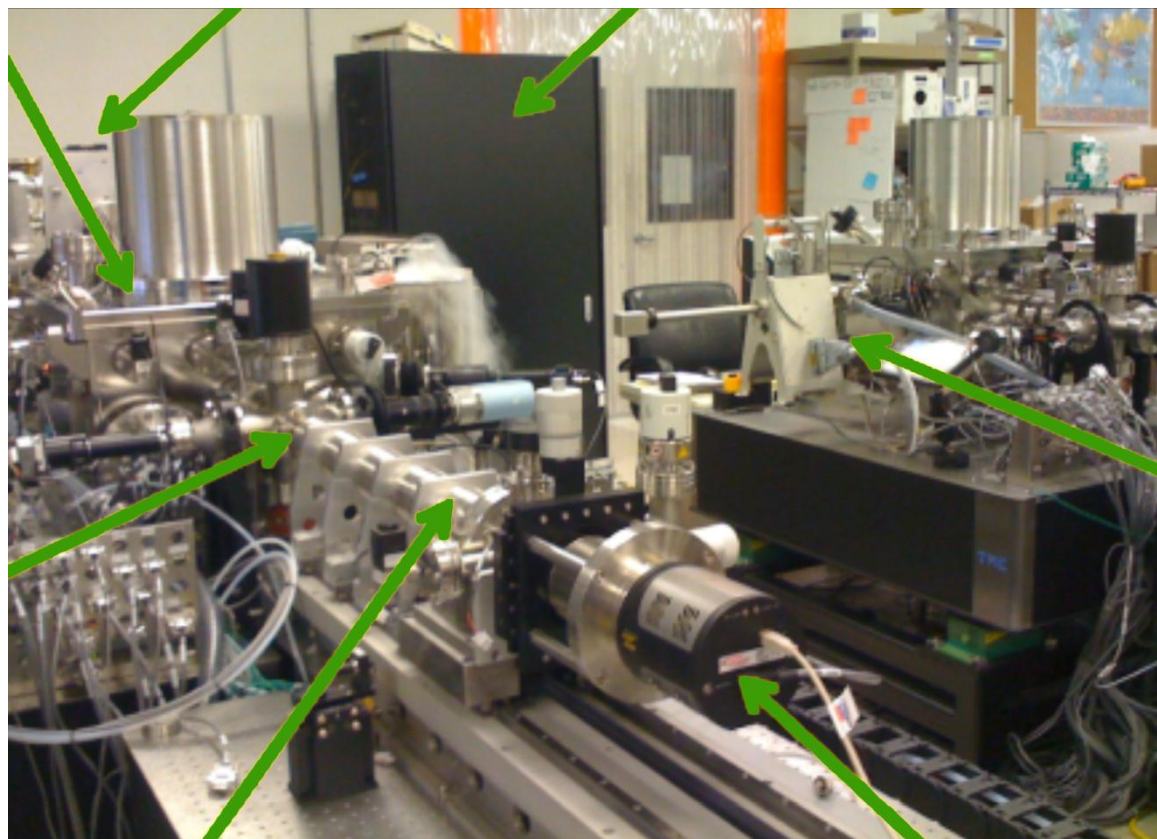
Lab-Based Cryo Soft X-ray Microscope

EQ-10SXR Light Source

Electronics Rack

Sample Chamber

X-ray Alignment System



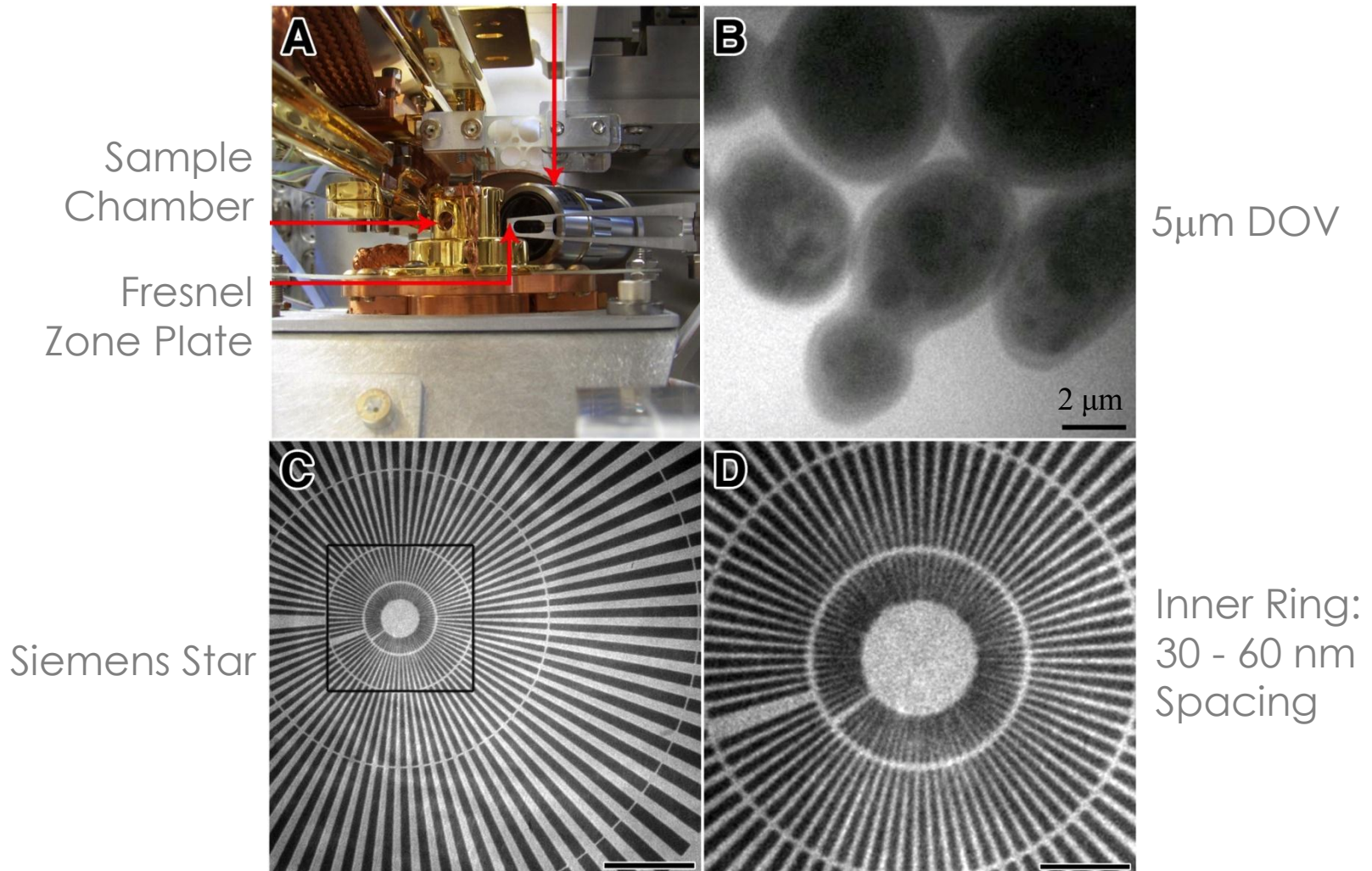
Integrated Cryo-Transfer System

Variable Magnification Slide

CCD Camera

Energetiq laboratory light source generates photons at an energy of 430 eV within the “water window”

Light Objective Alignment



Microscope achieves better than 50 nm resolution at 110 Kelvin

Carlson, D.B., Gelb, J., Palshin, V., and Evans, J.E. (2012) *Microscopy & Microanalysis* **In Press**

Radiograph Tilt Series



Tomogram



Segmented Volume

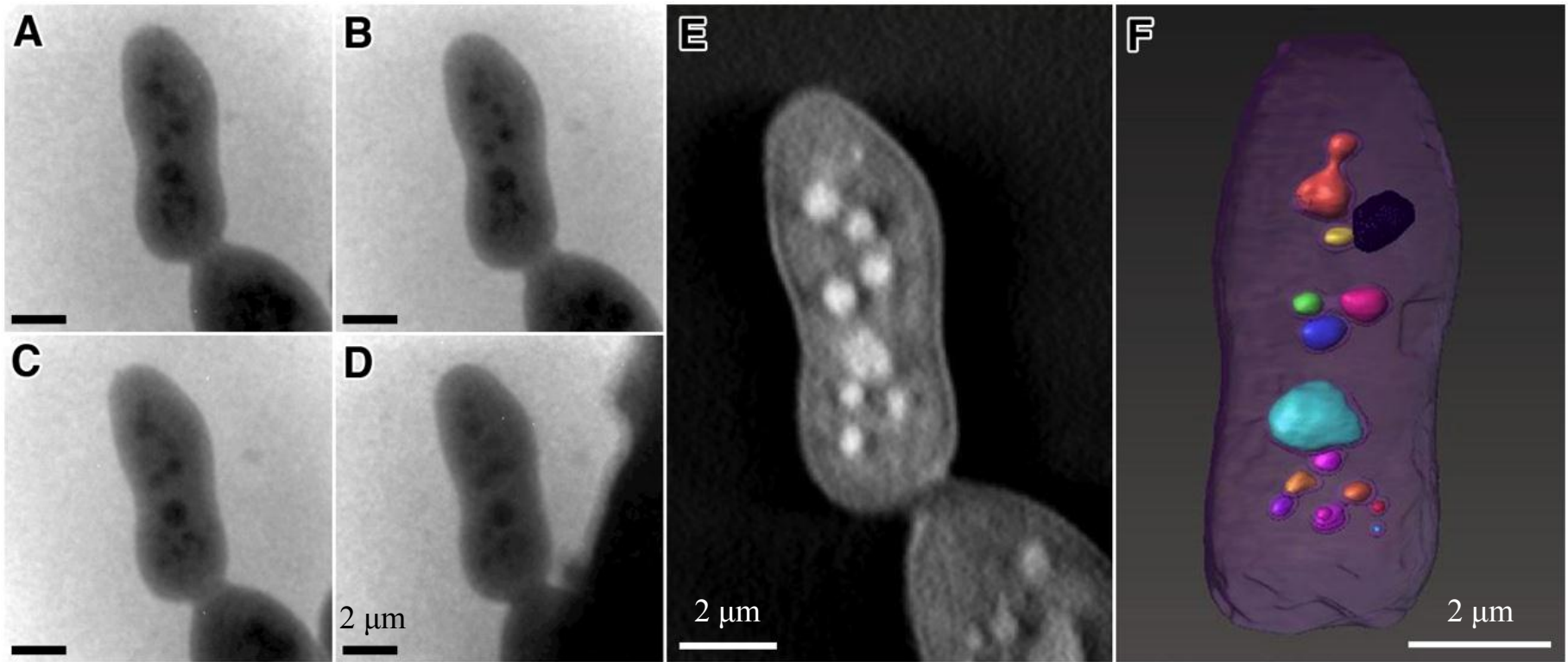


Cell membrane

Mitochondria

Nucleus

Vacuole

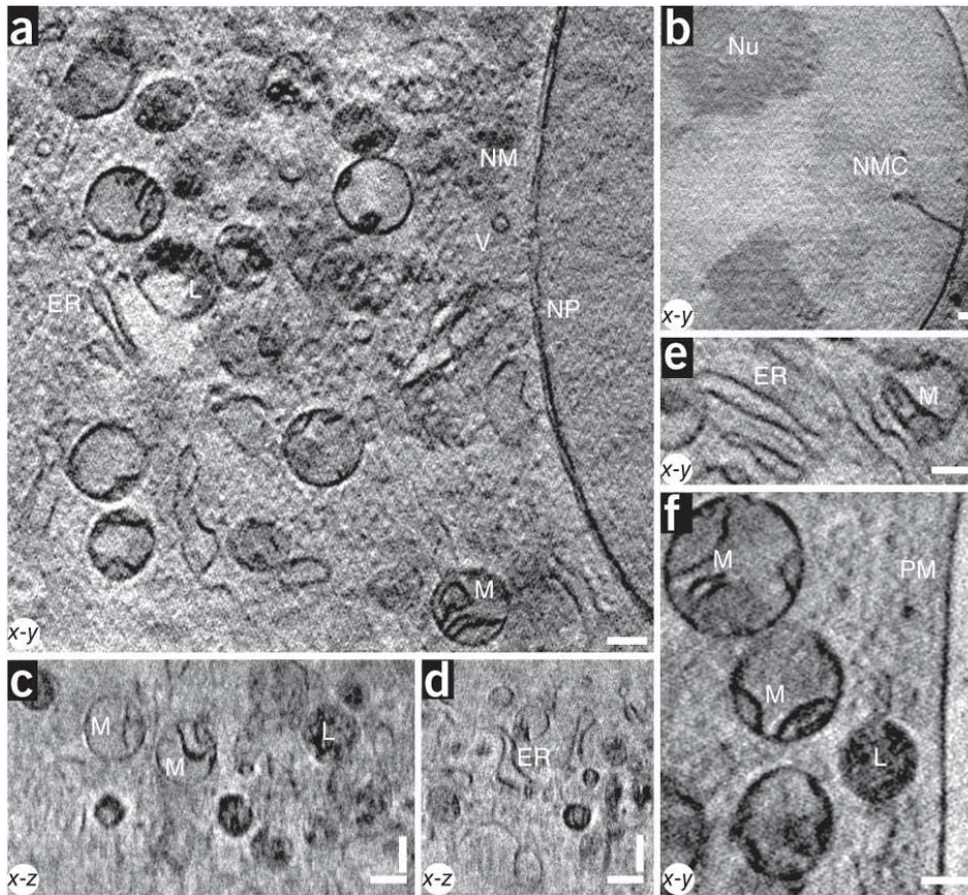


Smallest visible features at 90 nm diameter
using total dose of 7 MGy for tilt series.

3D resolution of 70 nm.

Cell membrane
Mitochondria
Nucleus
Vacuole

Resolution Better than 40 nm Permits Direct Classification of Cellular Structures



Structures Identified:

mitochondria (M),
lysosomes (L),
endoplasmic reticulum (ER),
vesicles (V),
plasma membrane (PM),
nuclear membrane (NM),
nuclear pores (NP),
nucleoli (Nu)
and nuclear membrane
channels (NMC).

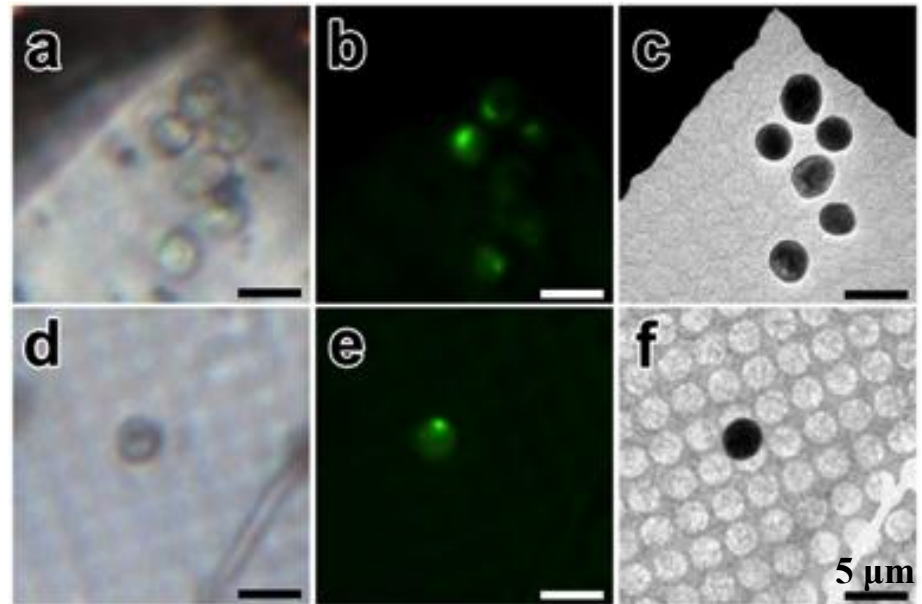
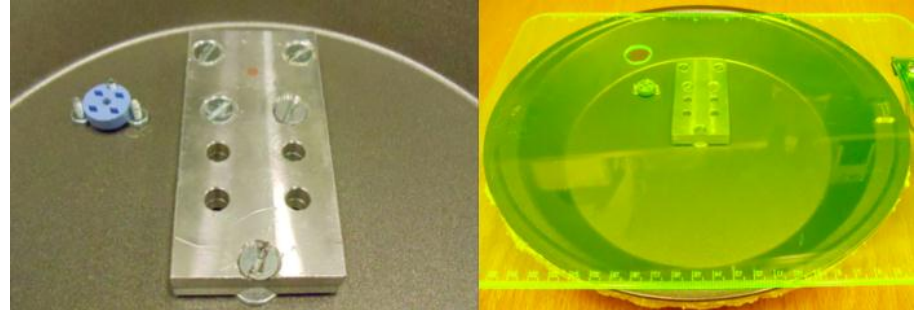
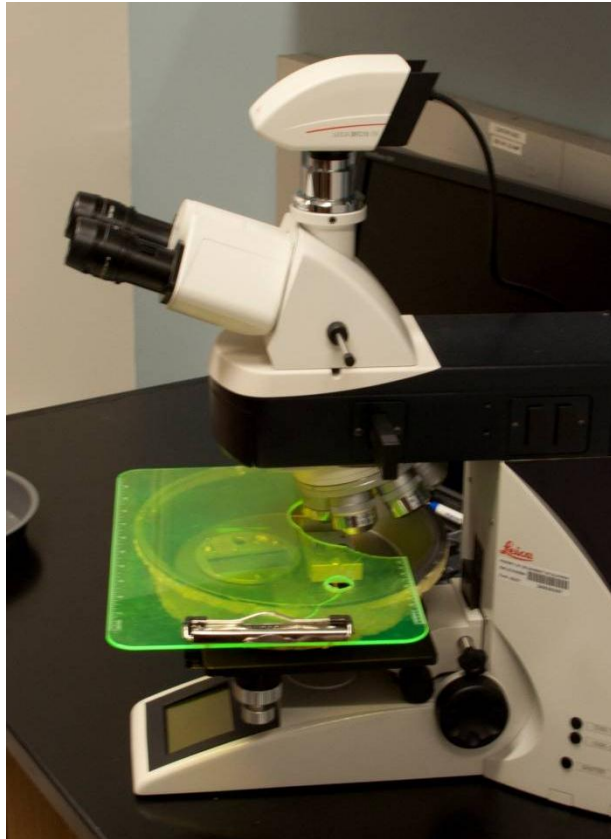
Schneider, G., et al., (2010) *Nature Methods* 7:985-987

Synchrotron generated tomogram at 36 nm resolution of mouse adenocarcinoma cell Imaged in frozen hydrated state with a total cumulative imaging dose of 1,000 MGy.

Until laboratory-based soft x-ray tomography can surpass the ~ 40 nm resolution threshold (3-D) other correlative approaches will be needed to enable unambiguous identification of visualized organelles.

The next few slides demonstrate the first results using correlative microscopy of the exact same cell with lab-based cryogenic fluorescence, soft x-ray and electron microscopy.

Correlative Cryogenic Light and Electron Microscopy

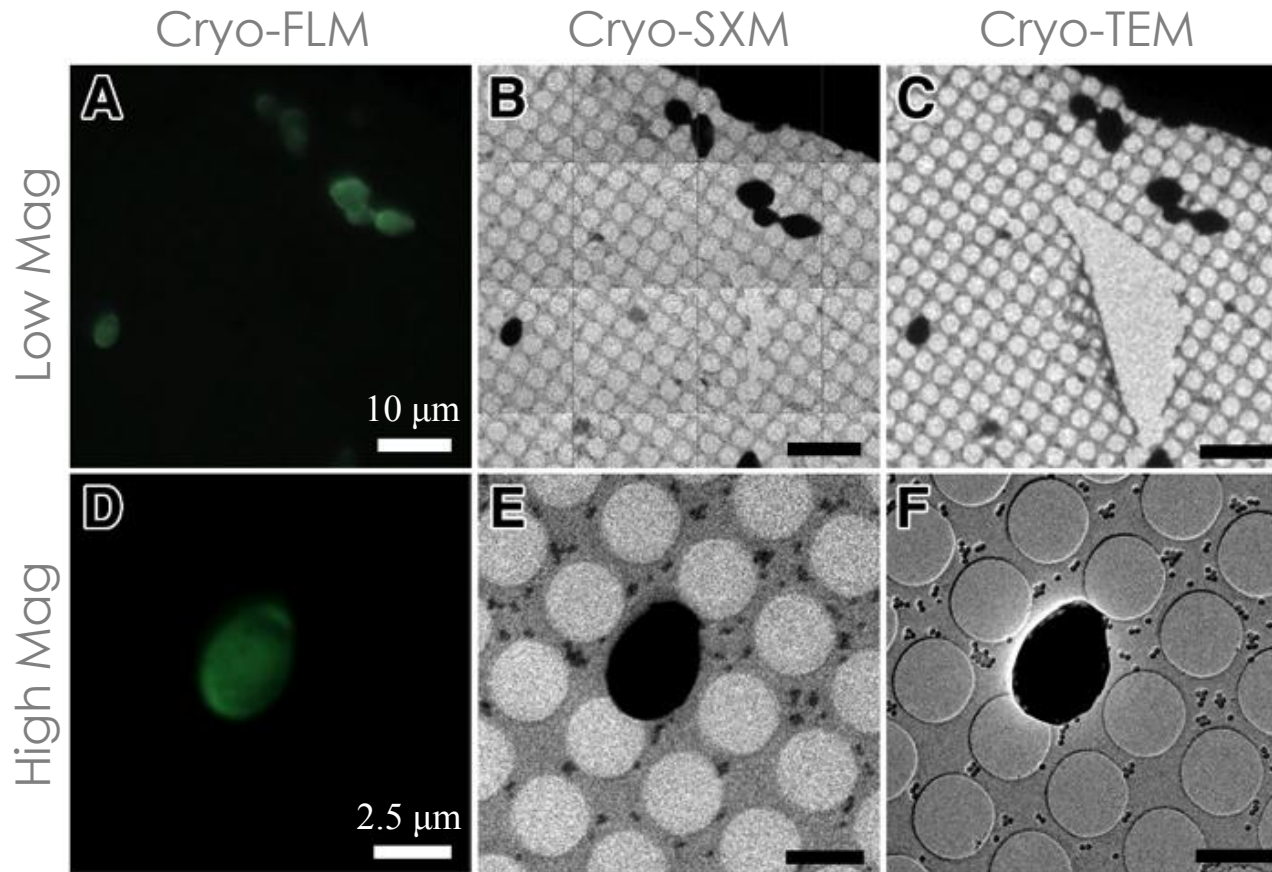


Cryo-LM

Cryo-FLM

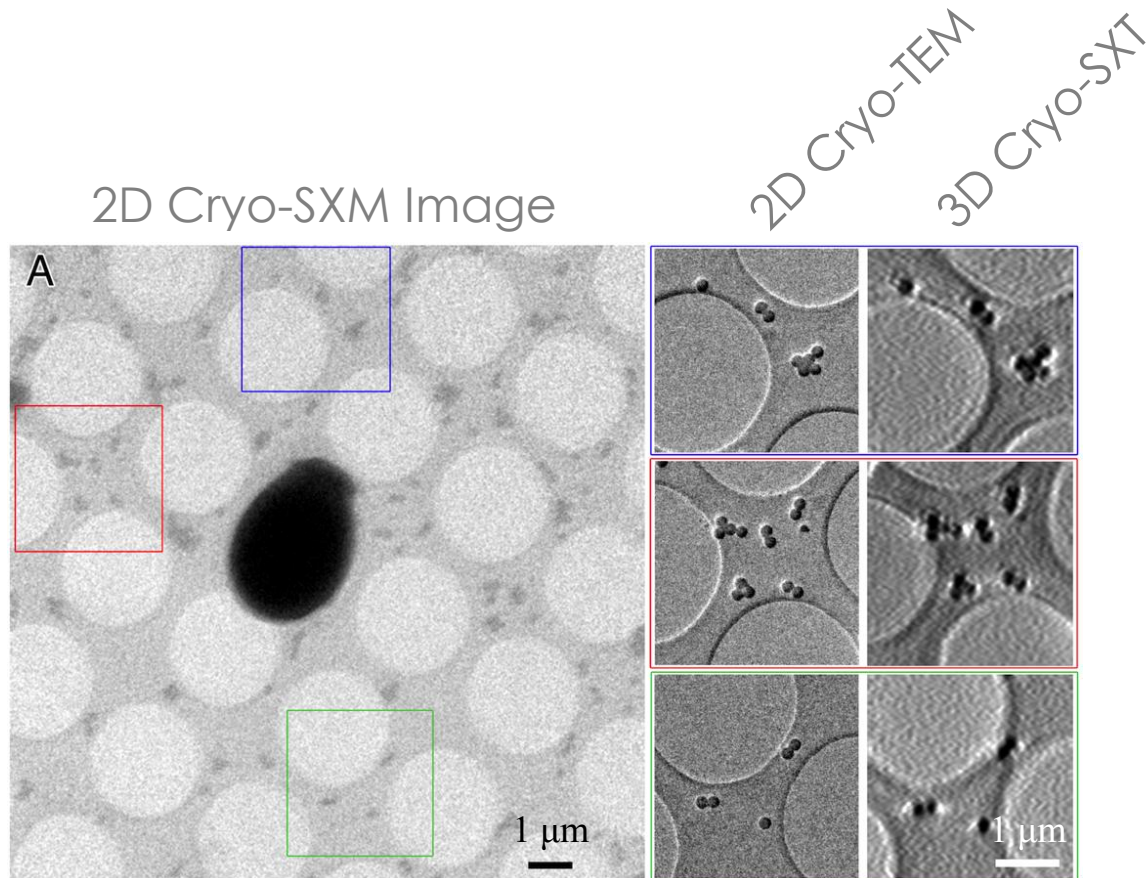
Cryo-TEM

Multi-Technique Correlative Cryogenic Microscopy



Comparative data of the exact same cell using cryogenic-
Fluorescence, soft X-ray and Electron Microscopy

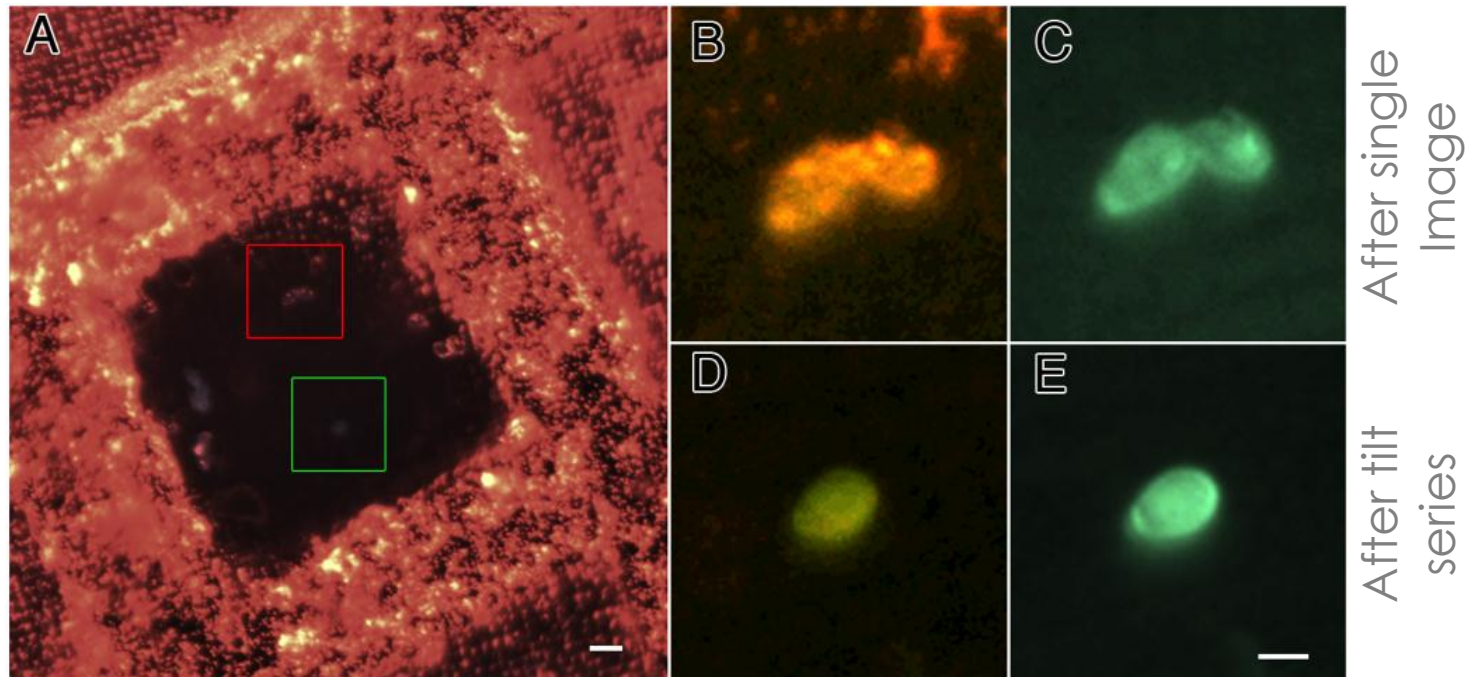
Fiducial Markers Can Help Align Datasets for Correlative Microscopy



Comparative data of the exact same 200 nm polystyrene beads.

Cryo-TEM resolution ~ 2 nm, Cryo-SXT resolution ~ 70 nm.

Some Fluorophores Could Allow Simultaneous Correlative Microscopy



YFP and Crimson Red Fluorophores photobleach.

However, eGFP fluorescence survives 7MGy x-ray irradiation during tilt series and could be used for simultaneous correlative imaging using the same instrument.

Current:

- Demonstrated laboratory-based cryogenic soft X-ray tomography for whole cell imaging.
 - Resolution and depth-of-view similar to a recent publication from Sweden (Hertz, H.M., et al. (2012) *J. Struct. Bio.* **177**(2), 267-272).
- The current platform is commercially available which should increase access to whole cell tomography.

Future Outlook:

- Integrate high magnification/N.A. objective lens for simultaneous 3D correlative light/fluorescence and x-ray microscopy.
- Incorporate future developments for improved laboratory light sources to increase the attainable spatial resolution and access even thicker specimens.

Thank you for your attention!

